## Supplementary Information

Label-Free Electrical Assay of Fibrous Amyloid  $\beta$  Based on Semiconductor Biosensing

Sho Hideshima,<sup>a</sup> Masumi Kobayashi,<sup>b</sup> Takeyoshi Wada,<sup>c</sup> Shigeki Kuroiwa,<sup>a</sup> Takuya Nakanishi,<sup>a</sup> Naoya Sawamura,<sup>c</sup> Toru Asahi<sup>ac</sup> and Tetsuya Osaka<sup>\*ab</sup>

 <sup>a</sup> Institute for Nanoscience & Nanotechnology, Waseda University, 513 Wasedatsurumaki-cho, Shinjuku-ku, Tokyo 162-0041, Japan
<sup>b</sup> Department of Nanoscience and Nanoengineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan
<sup>c</sup> Department of Life Science & Medical Bioscience, Waseda University, TWIns, 2-2

\* Fax: +81 3 3205 2074; Tel: +81 3 5286 3202; E-mail: osakatets@waseda.jp (T.O.).

Wakamatsu, Shinjuku-ku, Tokyo 162-8480, Japan

### 1. Materials

The amyloid proteins, A $\beta$ 42 and A $\beta$ 40, were purchased from Peptide Institute, Inc. and Congo red (CR) from Tokyo Chemical Industry Co., Ltd. The self-assembled monolayer reagent, 3-aminopropyltriethoxysilane (APTES), and human serum albumin (HSA) were purchased from Sigma–Aldrich Inc. The semiconductor-based field effect transistor (FET) biosensor was obtained from Toppan Printing Co., Ltd. The other chemicals were purchased from Kanto Chemical Co. Inc. The buffer, phosphate buffered saline (1× PBS) of pH 7.4, was made by using 137 mM NaCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>•12H<sub>2</sub>O, 2.7 mM KCl, and 1.5 mM KH<sub>2</sub>PO<sub>4</sub>. Diluted PBS, 0.01 × PBS (pH 7.4), was prepared by diluting 1× PBS with ultrapure water.

## 2. Preparation of amyloid beta (1-42) isoform

The amyloid  $\beta$  peptides, A $\beta$ 42 and A $\beta$ 40, were dissolved in 0.1% ammonia water, followed by the dilution with PBS, to obtain 100  $\mu$ M concentration. Subsequently, the solution was incubated for 0-3 days at 37°C in a 1.5 mL tube. Additionally, the sample of A $\beta$ 42 was diluted with PBS to a required concentration in the range of 100 fM to 100  $\mu$ M.

3. Functionalization of semiconductor FET-based device for amyloid  $\beta$  detection The surface of silicon dioxide as a gate insulating film of the semiconductor-based FET biosensor was exposed to O<sub>2</sub> plasma (200 W for 1 min) to introduce hydroxyl groups on the surface, followed by coating with SAM of APTES. First, the SAM was formed on the silicon dioxide surface by immersing in 1% (v/v) APTES in toluene at 60°C for 7 min in an argon atmosphere. After the SAM modification, for cross-linker, glutaraldehyde (GA) was allowed to react with the amino-terminated surface by immersing a gate area of the aminopropylsilane (APS)-modified FET in a solution of 2.5% GA in 1× PBS for 30 min. Subsequently, the probe molecule CR was allowed to react with the aldehyde moiety of GA-modified surface for 60 min. This reaction resulted in the fabrication of the CR-immobilized FET. The gate voltage ( $V_g$ ) - drain current ( $I_d$ ) relation of the CR-immobilized FET was measured and used as the reference. The measurements were made in the dark with a semiconductor parameter analyzer (2612A, Keithley Instruments Inc., USA) at room temperature in 0.01× phosphate buffered saline (PBS, pH 7.4) by sweeping the  $V_g$  from -3 V to 1 V with a 0.1 V drain voltage. The reference electrode was Hg/Hg<sub>2</sub>SO<sub>4</sub>. The CR-immobilized FETs were immersed in the A $\beta$  solutions (A $\beta$ 42 and A $\beta$ 40), which were prepared by following the above-mentioned procedure, for 30 min. After the immersion, the residue was washed with 1× PBS, followed by 0.01× PBS. The  $V_g$  -  $I_d$  characteristic of the A $\beta$ reacted FET was measured in 0.01× PBS and compared with the reference. The threshold voltage shift ( $\Delta V_g$ ) was calculated.

### 4. Optimization and characterization of the CR-immobilized surface

The state of the immobilization of CR molecule on the surface of SiO<sub>2</sub> substrate was characterized by XPS. XPS measurements were performed on a spectrophotometer (PHI-5000 Versa Probe WS, ULVAC-PHI Inc.) using an Al K $\alpha$  X-ray source. The three specimens were examined: the specimens treated with the CR solution of either 10 ng/mL or 1 µg/mL, hereafter denoted as "10 ng/mL CR" and "1 µg/mL CR", respectively, and the specimen without CR treatment, denoted as "GA-modified". As compared with the "GA-modified" specimen, the increase in the peak intensity was

observed for both C 1s and N 1s regions after the treatment with CR. Here the increment from "GA-modified" state for "1  $\mu$ g/mL CR" was greater than that for "10 ng/mL CR". For the S 2p region, a weak but a certain component was observed at the binding energy between 167 and 169 eV for "1  $\mu$ g/mL CR", which was not observed for "GA-modified". Here, a very weak S 2p component appeared at the same binding energy for "10 ng/mL CR". Considering the chemical structure of CR molecule, those are attributable to the immobilization of CR molecules on the surface.



Figure S1. X-ray photoelectron spectra of (a) C 1s, (b) N 1s, and (c) S 2p regions of GA-modified SiO<sub>2</sub> specimens before (blue, "GA-modified") and after the treatment with CR solution (red, "10 ng/mL CR" and green, "1  $\mu$ g/mL CR").

In our preliminary study, we confirmed that the concentration of congo red (CR) solution for immobilization have an effect on the degree of interaction between CR molecules and yeast prion Sup35NM.<sup>1</sup> After the experiments, 10 ng/mL CR was determined to be the optimal concentration for CR immobilization of the FET gate surface. The signals decrease as the CR concentration is increased by more than 10 ng/mL, which is assumed to be related to the formation of helical aggregates of CR molecules at higher concentrations through intermolecular  $\pi$ - $\pi$  interactions in solution, like dimers or oligomers, impairing the binding specificity of the aggregated CR probe to the fibril proteins.

# Reference

 S. Hideshima, S. Wustoni, S. Kuroiwa, T. Nakanishi, A. Koike and T. Osaka, ChemElectroChem, 2014, 1, 51-54.